RESEARCH ARTICLE

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miR-98-5p promotes apoptosis and inhibits migration and cell growth in papillary thyroid carcinoma through Bax/Caspase-3 by HMGA2

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Abstract

Background: The aims of this study were to investigate the function and mechanism of miRNA-98-5p in papillary thyroid carcinoma.

Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure the expression of miRNA-98-5p in papillary thyroid carcinoma. Western blotting and caspase-3/9 activity levels, flow cytometric analysis, cell migration assays, DAPI assay, cell proliferation assay, and LDH activity levels were used in this study.

Results: In patient with papillary thyroid carcinoma, miRNA-98-5p was reduced, and HMGA2 was increased. Downregulation of miRNA-98-5p promoted the cell growth, inhibited apoptosis, and induced HMGA2 protein expression in papillary thyroid carcinoma cell via activation of HMGA2. Overexpression of miRNA-98-5p inhibited the cell growth, induced apoptosis, and suppressed HMGA2 protein expression in papillary thyroid carcinoma cell through the suppression of HMGA2. Si-HMGA2 inhibited the effects of anti-miRNA-98-5p on cell growth of papillary thyroid carcinoma.

Conclusion: Therefore, these results suggested the regulation of HMGA2 suppresses proliferation of papillary thyroid carcinoma through miRNA-98-5p.

KEYWORDS
apoptosis, HMGA2, miRNA-98-5p, papillary thyroid carcinoma

1 | INTRODUCTION

Thyroid carcinoma is one of the common head and neck malignancies.[1] It is also the most common malignancy in endocrine system, which accounts for about 1% of systemic malignancies.[1] Thyroid carcinoma is more commonly seen in female, with the male-to-female ratio of 1:2.58.[2] It can occur in all age groups from children to the elderly. This is different from general cancers that frequently occur in the elderly. Specifically, thyroid carcinoma is more commonly seen in young adults, with the average age of onset of about 40 years.[3] Papillary thyroid carcinoma (PTC) shows the pathological manifestation of thyroid mass with hard texture, either with single or multiple lesions.[3] Patients with multiple lesions have taken up 20%-65%. Morphologically, thyroid carcinoma may have enveloped or incomplete enveloped, with dark red section.[4] It is frequently accompanying with calcification, which leads to sense of scrubbing when cutting.[4] Alternatively, it can be a cystic and solid mass containing brown or dark brown liquid, with papilla on the cyst wall.[4]

Thyroid carcinoma can be classified into differentiated and nondifferentiated types according to its histological structure.[5] The differentiated type has accounted for about 90%, which is one of the malignancies with the most rapidly increased morbidity recently.[6]

Differentiated carcinoma can be further divided into PTC and follicular thyroid carcinoma (FTC).[5] PTC occupies 75% of all thyroid carcinoma cases, while FTC only takes up 16%.[5] Meanwhile, thyroid carcinoma can also be divided into parafollicular cell carcinoma (medullary carcinoma) and follicular epithelial cell carcinoma based on the origin cell. Most thyroid carcinoma cases derive from follicular epithelial cell.[1] On the other hand, follicular epithelial cell carcinoma includes papillary carcinoma, follicular carcinoma, and undifferentiated carcinoma.[6]

miRNA is a class of endogenous non-coding small RNA molecule. It generally targets one or more mRNAs and binds with its 3′-untranslated region (3′-UTR).[7] Thus, it can inhibit or degrade the target miRNA at translation level and negatively regulate target gene expression.[8] Thousands of miRNAs have been discovered at present. They can extensively regulate important biological processes, such as cell growth, proliferation, invasion, differentiation, and apoptosis.[9] An increasing number of studies have indicated that miRNA is aberrantly expressed in multiple malignancies, including PTC.[9] Moreover, miRNA displays superb stability in formalin-fixed tissue samples.[8] Therefore, the related expression profiles in tissue samples can be stably obtained. Research on miRNA expression profile indicates that miRNA is closely related to tumor genesis, progression and treatment response.[10] This indicates that it has the potential to serve as biomarker to be used in malignancy diagnosis and prognosis judgment.[10]

HMGA2 belongs to the high mobility protein family. It generally locates in the cell nucleus and shows no transcription activity. However, it can bind with DNA to change its chromosome structure, thus regulating gene transcription. As a result, it is also referred to as the structural transcription factor. It plays a vital role in chromosome remodeling and gene regulation. HMGA2 is highly expressed in the tissue samples from PTC patients. It is markedly correlated with lung cancer classification, metastasis, and patient survival.[11] The aims of this study were to investigate the function and mechanism of miRNA-98-5p in papillary thyroid carcinoma.

2 | MATERIALS AND METHOD

2.1 | Patient samples and quantitative real-time polymerase chain reaction

Serums of PTC patients were obtained from Fujian Medical University Union Hospital after surgical resection. Serums of PTC patients were obtained from Fujian Medical University Union Hospital. The study
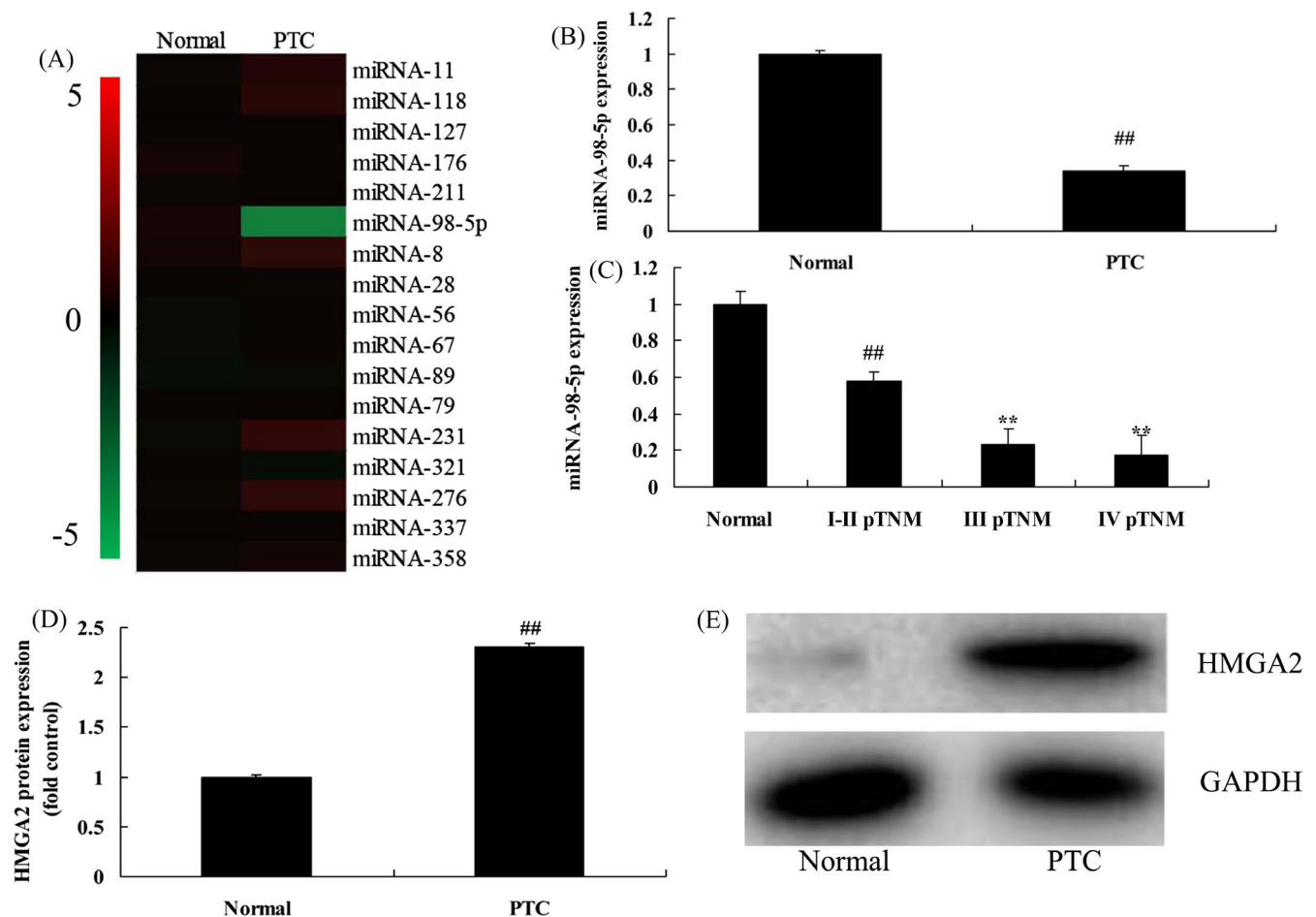

FIGURE 1 miRNA-98-5p and HMGA2 expression in papillary thyroid carcinoma. Gene chip and qPCR for miRNA-98-5p (A and B), HMGA2 protein expression (C), statistical analysis of HMGA2 protein expression (D). Normal, normal group; PTC, papillary thyroid carcinoma group
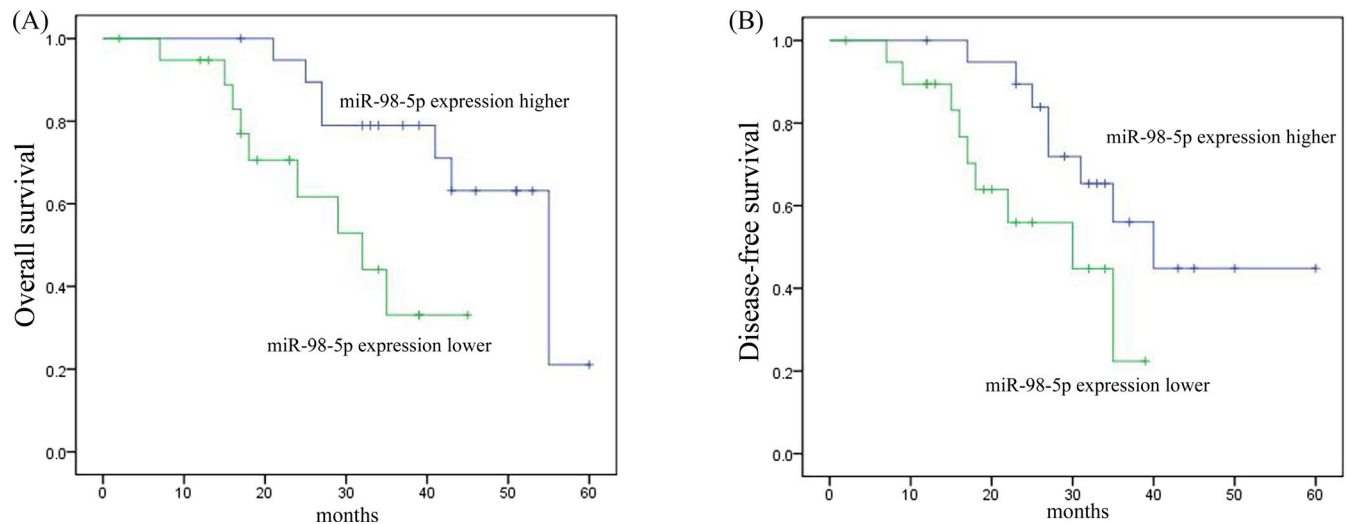
FIGURE 2 Relationship with OS/DFS of patient and miRNA-98-5p expression. Relationship with OS (A)/DFS (B) of patient and miRNA-98-5p expression

protocol was approved by the Institute Research Ethics Committee at Fujian Medical University Union Hospital. Total RNA was extracted from the serum and cell using Trizol (Invitrogen). cDCA was reverse-transcribed using the TaqMan MiRNA Reverse Transcript Kit (Applied Biosystems). miRNAs were quantified using SYBR Premix Ex TaqTM (TaKaRa) by ABI 7500 Fast Sequence Detection System (Applied Biosystems Prism) and the relative expression using the 2-ΔΔCT method. U6 forward, 5′-AGAGCCTGTGGTGTCCG-3′, reverse, 5′-CATCTTCAAAGCACTTCCCT-3′.

2.2 | Cell culture

Human PTC cell line A-PTC cell was cultured in the RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), 1% penicillin G (100 units/mL), and 1% streptomycin (100 μg/mL) at 37°C in a humidified atmosphere of 5% CO_2.

2.3 | Transfection and luciferase assay

Si-HMGA2, miRNA-98-5p, anti-miRNA-98-5p mimic, and corresponding negative control (miR-NC) were purchased from GenePharma Co., Ltd. Mimic or plasmids were transfected into K1 cell using Lipofectamine 2000 (Invitrogen).

2.4 | Cell proliferation assay and LDH activity levels

Cells (1 × 104 cells per well) were plated in 96-well plates, and MTT was added to cell cultures at a final concentration of 5 mg/mL 4 hours at 37°C. DMEM was removed, and DMSO was added into cell for 20 minutes at 37°C. Absorbance was measured at 570 nm using an ELISA reader (Thermo Labsystems).

After transfection of 48 hours, cell was used to measure the LDH activity levels using LDH activity kits. Absorbance was measured at 450 nm using an ELISA reader (Thermo Labsystems).

2.5 | Cell migration assays and DAPI assay

Cell migration was evaluated using a wound-healing assay and cultured in 24-well plates (1 × 104 cells per well). Cells were seeded into the upper chamber of Matrigel-coated inserts with free-serum medium. DMEM with 20% FBS was added to the lower chamber as chemoattractant. After 48 hours, cells invaded to the lower surface of filter and were fixed with ice-70% ethanol for 30 minutes. Cell was stained with 0.1% crystal violet for 10 minutes and counted in five randomly selected fields using an X71 inverted microscope (Olympus).

After transfection of 48 hours, cell was washed with PBS and fixed with ice-70% ethanol for 30 minutes. Cell was stained with DAPI assay for 30 minutes at darkness and washed with PBS. Cell was observed using an X71 inverted microscope (Olympus).

2.6 | Flow cytometric analysis

Cells (1 × 106 cells per well) were plated in 6-well plates and washed with PBS for 15 minutes. 5 μL of Annexin V-FITC/PI was added into cell for 15 minutes at darkness. Subsequently, the results were analyzed by flow cytometry (c6) using FloMax software.

2.7 | Western blotting and caspase-3/9 activity levels

Cellular proteins were extracted in RIPA buffer (Biomed), and protein concentration was assayed using the BCA Protein Assay Reagent Kit (Biomed, China). 50 μg of equal amounts was separated by electrophoresis on a 10% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. Membrane was blocked with 5% nonfat in TBST for 1 hours at 37°C and incubated overnight (4°C) with the primary antibody: Bax, HMGA2, and GAPDH. Subsequently, the membranes were incubated with goat anti-mouse for 1 hours at 37°C after washing for 15 minutes using
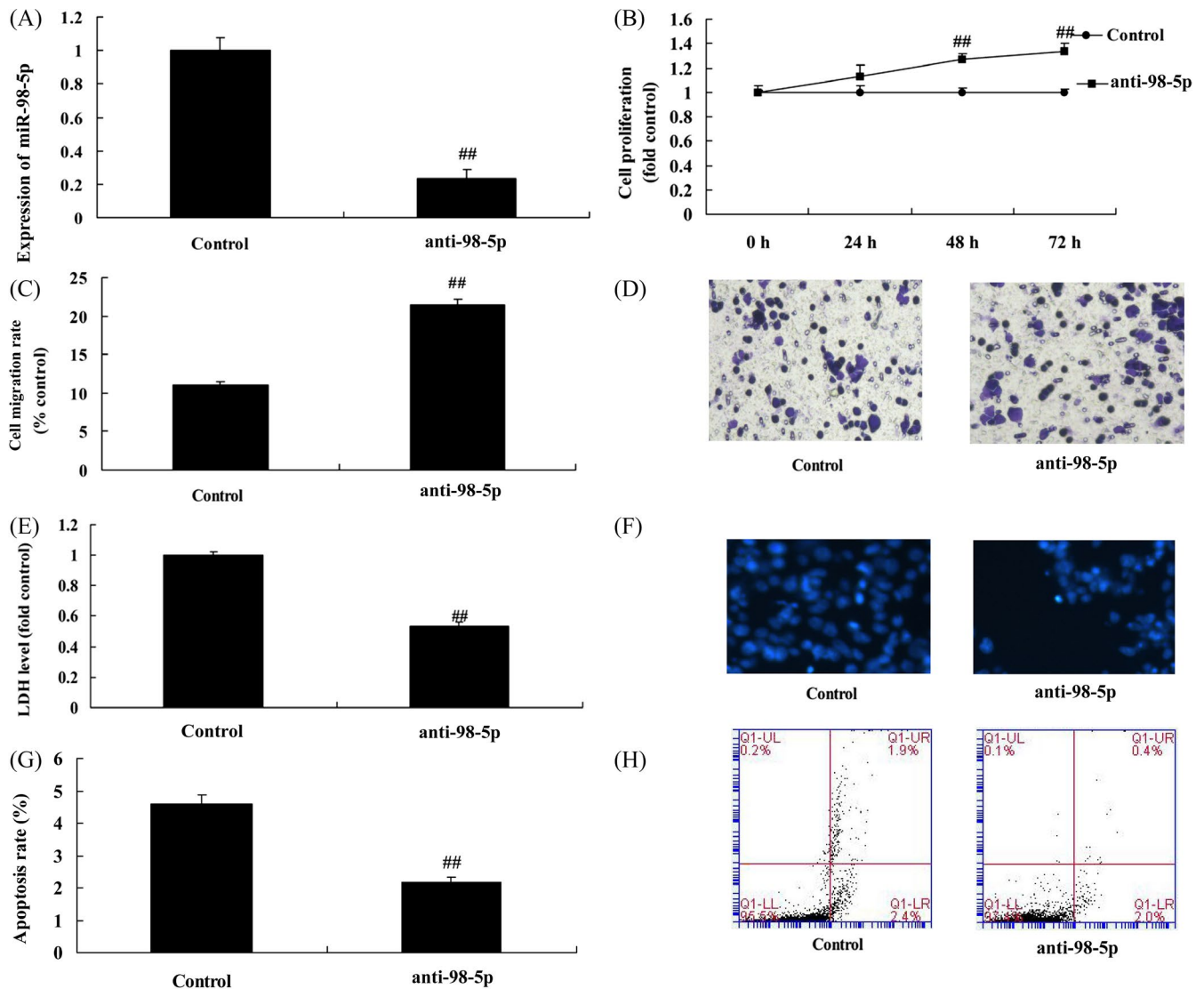
FIGURE 3 Downregulation of miRNA-98-5p on cell growth of papillary thyroid carcinoma. miRNA-98-5p expression (A), cell growth (B), cell transfer (C and D), LDH level (E), DAPI assay (F), apoptosis rate (G and H). Control, control negative group; Anti-98-5p, downregulation of miRNA-98-5p group. ##$P < .01$ vs control group

TBST. Finally, protein bands were detected by chemiluminescence using the Amersham ECL Plus Western Blotting Detection Kit (GE Healthcare Bio-Sciences) and analyzed using Image-ProPlus 6.0 software (Media Cybernetics, IncSA).

Cellular proteins were extracted in RIPA buffer (Biomed), and protein concentration was assayed using the BCA Protein Assay reagent kit (Biomed). 50 μg of equal amounts was used to measure caspase-3/9 activity levels using caspase-3/9 activity levels kits. Absorbance was measured at 405 nm using an ELISA reader (Thermo Labsystems).

2.8 | Statistical analysis

All results were expressed as mean values ± standard deviation (n ± 3). Experimental data were analyzed using one-way analysis of variance (ANOVA) and Tukey's post-test. $P < .05$ was considered as statistically significant.

3 | RESULTS

3.1 | miRNA-98-5p and HMGA2 expression in papillary thyroid carcinoma

Firstly, we analyzed the changes of miRNA-98-5p and HMGA2 expression in papillary thyroid carcinoma. Figure 1A,B shows that miRNA-98-5p expression was inhibited in papillary thyroid carcinoma, compared with the control group. Figure 1C,D shows that HMGA2 expression was inhibited in papillary thyroid carcinoma, compared with control group.

3.2 | Relationship with OS/DFS of patient and miRNA-98-5p expression

Next, we analyzed the role of miRNA-98-5p on OS and DFS of patient with papillary thyroid carcinoma. As shown in Figure 2A,B, OS
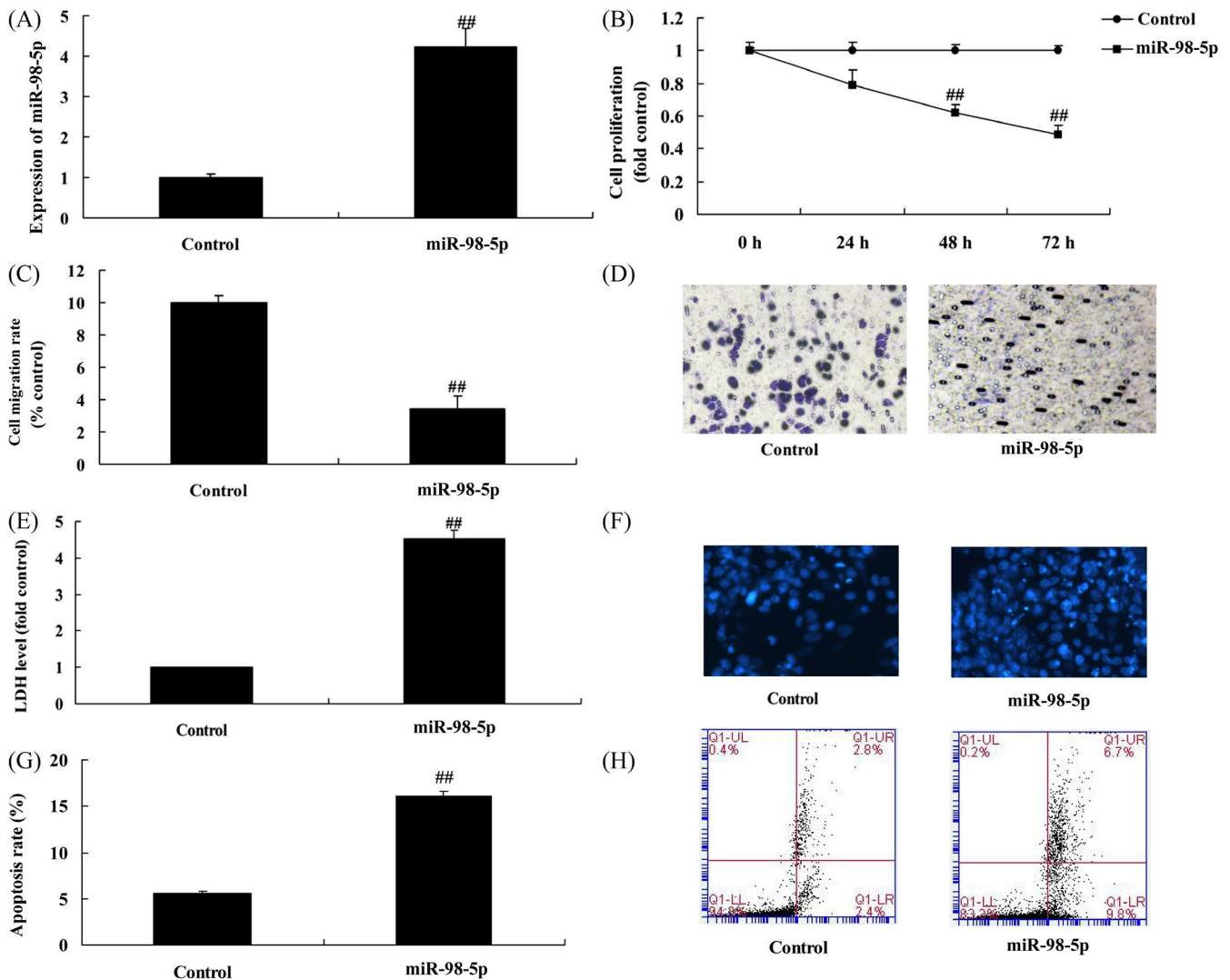
FIGURE 4 Overexpression of miRNA-98-5p on cell growth of papillary thyroid carcinoma. miRNA-98-5p expression (A), cell growth (B), cell transfer (C and D), LDH level (E), DAPI assay (F), apoptosis rate (G and H). Control, control negative group; miRNA-98-5p, overexpression of miRNA-98-5p group. ##P < .01 vs control group

and DFS of patient with miRNA-98-5p high expression were higher than those of patient with miRNA-98-5p low expression. These results showed that miRNA-98-5p participated in the development and progression of papillary thyroid carcinoma.

3.3 | Effects of miRNA-98-5p on cell growth of papillary thyroid carcinoma

Then, we analyzed the function of miRNA-98-5p in papillary thyroid carcinoma. Anti-miRNA-98-5p mimics were used to decrease the expression of miRNA-98-5p in vitro model of papillary thyroid carcinoma, compared with negative group (Figure 3A). Downregulation of miRNA-98-5p promoted the cell growth and cell transfer, and inhibited LDH level and apoptosis rate in vitro model of papillary thyroid carcinoma, compared with negative group (Figures 3B2H). Next, we evaluated the anti-cancer effects of miRNA-98-5p in papillary thyroid carcinoma. miRNA-98-5p mimics increased the expression of miRNA-98-5p in vitro model of papillary thyroid carcinoma, compared

with negative group (Figure 4A). Overexpression of miRNA-98-5p reduced the cell growth and cell transfer and induced LDH level and apoptosis rate in vitro model of papillary thyroid carcinoma, compared with negative group (Figures 4B3H).

Effects of miRNA-98-5p regulate HMGA2 in papillary thyroid carcinoma. In order to investigate the mechanism of miRNA-98-5p on apoptosis in papillary thyroid carcinoma, we firstly analyzed the effects of miRNA-98-5p on signaling pathway to affect papillary thyroid carcinoma cell growth. As shown in Figure 5A, HMGA2 expression was reduced in overexpression of miRNA-98-5p, compared with the negative group. miRNA-98-5p regulates the expression of HMGA2 by directly targeting its mRNA 3′-UTR (Figure 5B). Luciferase assay activity levels were reduced by overexpression of miRNA-98-5p, compared with negative group (Figure 5C). Overexpression of miRNA-98-5p suppressed the HMGA2 protein expression in papillary thyroid carcinoma, compared with negative group (Figure 5D). Overexpression of miRNA-98-5p suppressed the HMGA2 and Bax protein expression, and caspase-3/9 activity
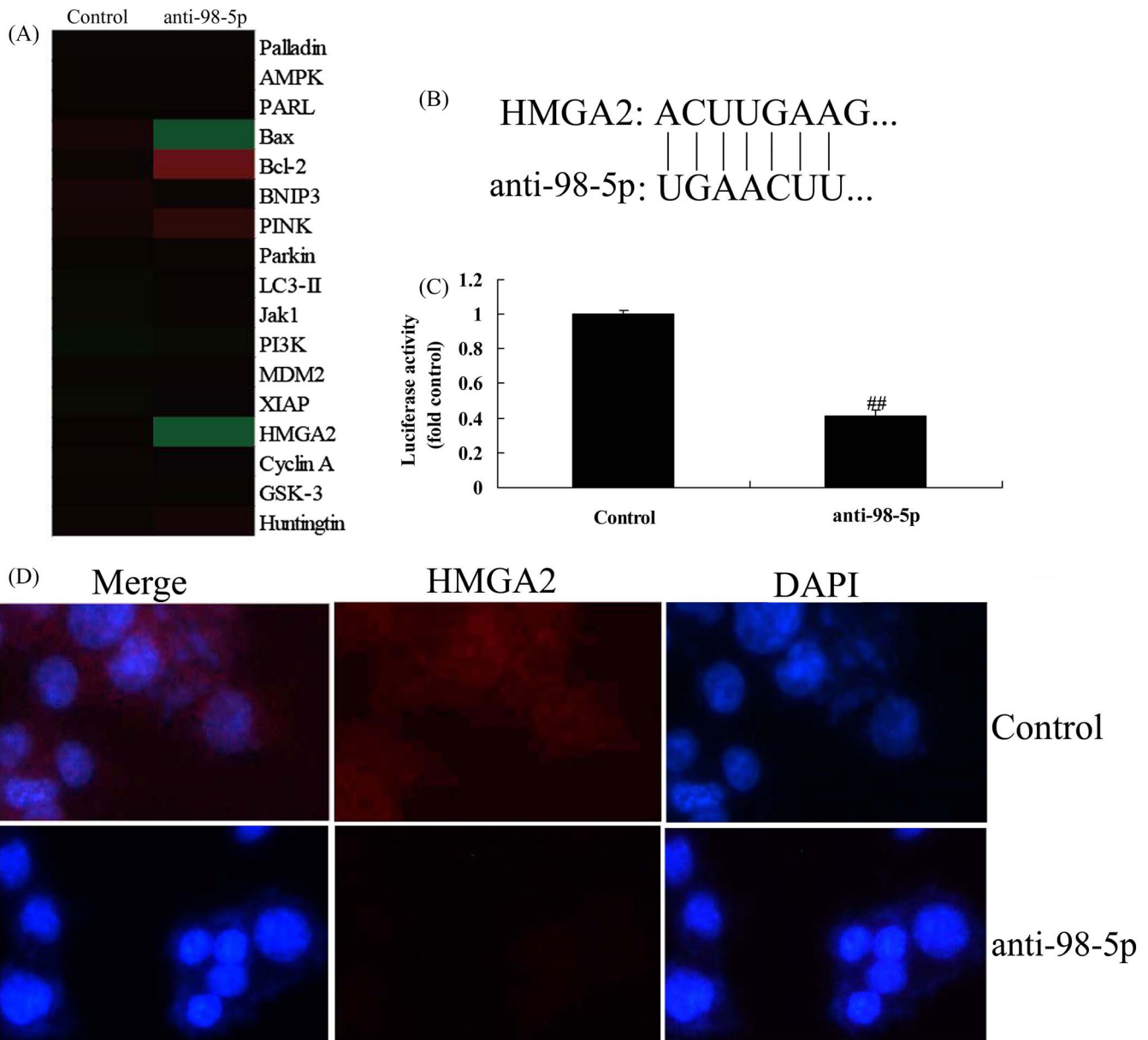
FIGURE 5 miRNA-98-5p regulates HMGA2 in papillary thyroid carcinoma. Gene chip (A), HMGA2 by directly targeting its mRNA 3′-UTR (B), luciferase assay activity levels (C), HMGA2 protein expression (D)

levels in papillary thyroid carcinoma, compared with negative group (Figure 6A-6E). Downregulation of miRNA-98-5p induced HMGA2 and Bax protein expression, and caspase-3/9 activity levels in papillary thyroid carcinoma, compared with negative group (Figure 6F-6J).

3.4 | Si-HMGA2 inhibited the effects of miRNA-98-5p on cell growth of papillary thyroid carcinoma

To further investigate the role of HMGA2 in the effects of anti-miRNA-98-5p on cell growth of papillary thyroid carcinoma. As shown in Figure 7A-7E, si-HMGA2 suppressed the HMGA2 and Bax protein expression, and caspase-3/9 activity levels in papillary thyroid carcinoma by miRNA-98-5p, compared with miRNA-98-5p

group. The inhibition of HMGA2 the effects of miRNA-98-5p on the promotion of cell growth and cell transfer, and inhibition of LDH level and apoptosis rate in papillary thyroid carcinoma by miRNA-98-5p, compared with miRNA-98-5p group (Figure 7F-7L).

4 | DISCUSSION

Papillary thyroid carcinoma accounts for about 60% of adult thyroid carcinoma cases and 100% of children thyroid carcinoma cases.[12] It has low malignant grade. PTC frequently occurs in young women, especially those aged 30-45 years.[6] About 80% tumors have multiple center, and approximately 1/3 involve the bilateral thyroid glands. It has neck lymph node metastasis at an early stage, but with favorable
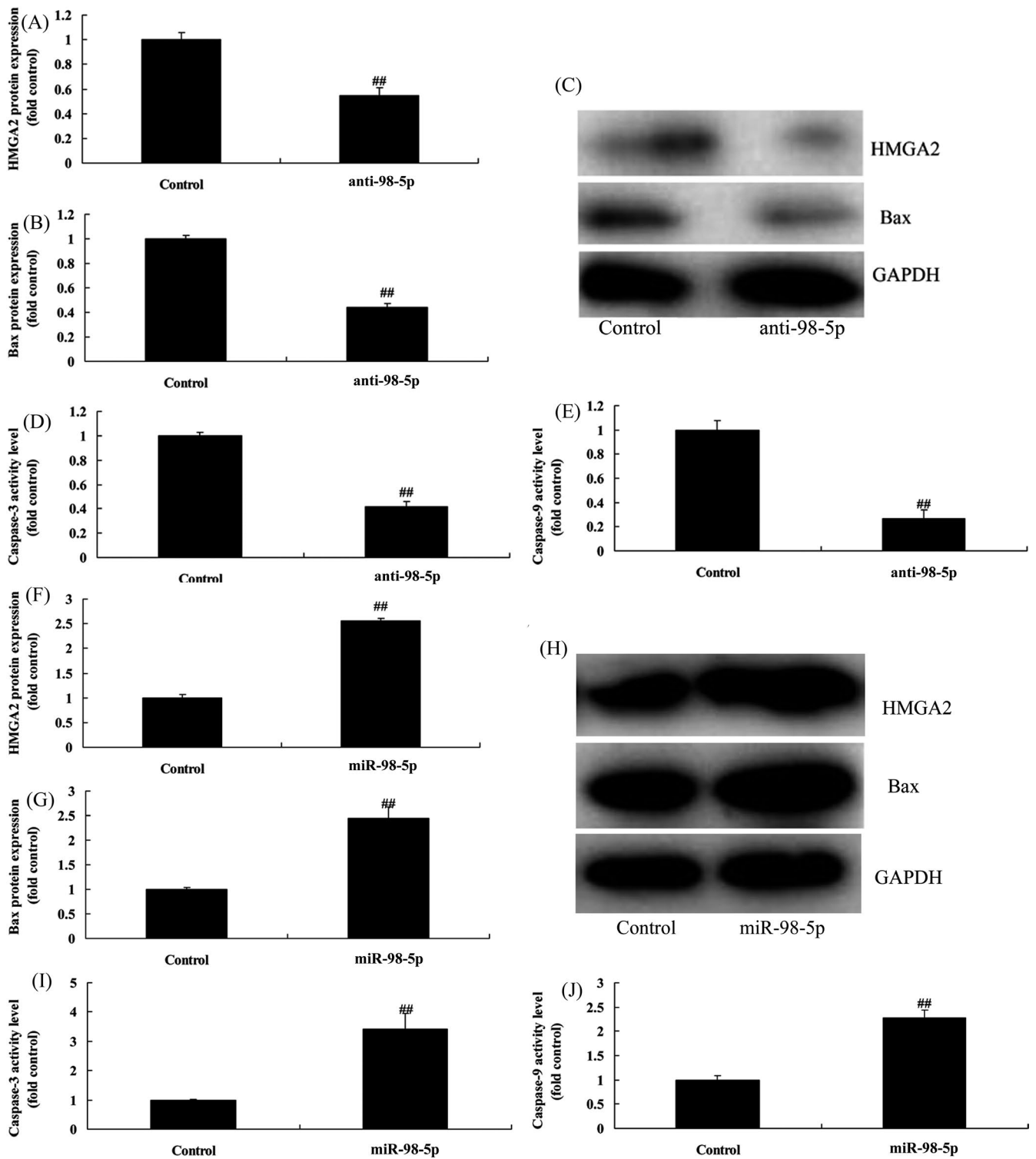
FIGURE 6 miRNA-98-5p regulates HMGA2/Bax protein expression in papillary thyroid carcinoma. HMGA2 and Bax protein expression (A) statistical analysis; HMGA2 and Bax protein expression (B and C) by Western blotting; caspase-3 and caspase-9 activity levels (D and E) following overexpression of miRNA-98-5p; HMGA2 and Bax protein expression (F) statistical analysis; HMGA2 and Bax protein expression (G and H) by Western blotting; caspase-3 and caspase-9 activity level (I and J) following downregulation of miRNA-98-5p. Control, control negative group; miRNA-98-5p, overexpression of miRNA-98-5p group; anti-98-5p, downregulation of miRNA-98-5p group. ##P < .01 vs control group

prognosis.[13] In the present study, we found that miRNA-98-5p expression was inhibited in papillary thyroid carcinoma, compared with control group.

Fu et al suggest that downregulated miR-98-5p promotes pancreatic ductal adenocarcinoma proliferation and metastasis by reversely regulating MAP4K4. {Fu, 2018 #3848}
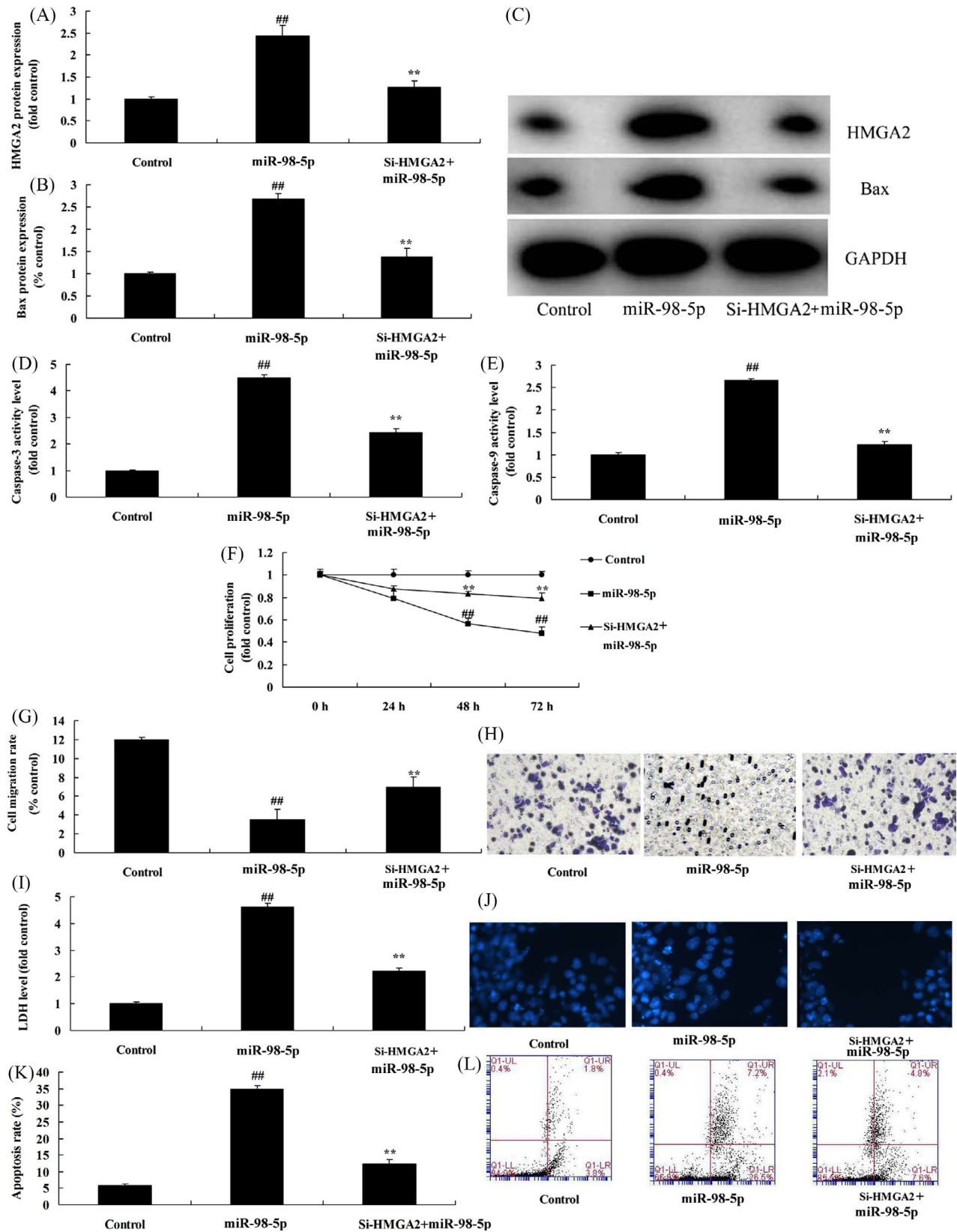
FIGURE 7 Si-HMGA2 inhibited the effects of miRNA-98-5p on cell growth of papillary thyroid carcinoma. HMGA2 and Bax protein expression (A) statistical analysis; HMGA2 and Bax protein expression (B and C) by Western blotting; caspase-3 and caspase-9 activity levels (D and E); cell growth (F) cell transfer (G and H); LDH level (I); DAPI assay (J); apoptosis rate (K and L). Control, control negative group; miRNA-98-5p, overexpression of miRNA-98-5p group; Si-HMGA2, overexpression of miRNA-98-5p and Si-HMGA2 group. ##P < .01 vs control group, **P < .01 vs control group

miRNA is highly conserved among different species. This has further increased its value in numerous important biological processes, including the differential diagnosis of PTC. This is an important discovery for PTC. miRNA is generally downregulated in tumor, but it is aberrantly upregulated in PTC.[14] This is verified in subsequent reports.[14] Therefore, we found that OS and DFS of patient with miRNA-98-5p expression lower were lower than those of patient with miRNA-98-5p expression higher.

HMGA2 is a structural transcription factor that involves in gene transcription regulation, chromatin condensation, and DNA damage repair.[11] It affects cell proliferation, growth, differentiation, aging, and death.[11] HMGA2 is found in virus-transformed rat thyroid cell line and is thus first linked with tumor.[15] Currently, mutation produced from HMGA2 gene rearrangement is considered to be related to benign tumor.[15] In contrast, the overexpression of full-length protein is related to the genesis and development of malignancies.[16] This study showed that the downregulation of miRNA-98-5p promoted the cell growth, inhibited apoptosis, and induced HMGA2 protein expression in papillary thyroid carcinoma cell via activation of HMGA2.

HMGA2 is a non-histone chromosomin that shows no transcription activity.[17] It can regulate the transcription of other genes through changing the chromatin structure.[18] HMGA2 is lowly expressed or not expressed in normal tissue, but upregulated during embryogenesis and in malignant tumor tissue.[17] Recent research finds that HMGA2 may participate in epithelial-mesenchymal transition, thus promoting tumor invasion and metastasis.[17,18] Patients with upregulated HMGA2 expression in tumor specimens and serum have dismal prognosis.[16] In some malignancies, HMGA2 can serve as a molecular marker for diagnosis or an independent factor for judging prognosis.[16] Our study suggested Si-HMGA2 inhibited the effects of anti-miRNA-98-5p on cell growth of papillary thyroid carcinoma.

In summary, in this study we report that the regulation of HMGA2 suppresses proliferation of papillary thyroid carcinoma through miRNA-98-5p. In conclusion, this study revealed that miRNA-98-5p profiles played a key role in papillary thyroid carcinoma, served as a novel and promising biomarker for diagnosis and disease severity of miRNA-98-5p.

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